APPENDIX A

CURRICULUM VITAE

NAME: Dr Heri A Bustamante

WORK ADDRESS: Sydney Water, 115-123 Bathurst Street Sydney NSW 2000 Australia

PRESENT POSITION: Project Manager

TELEPHONE: 02 9350 6817

E-MAIL ADDRESS: Heri.Bustamante@sydneywater.com.au

EDUCATION AND QUALIFICATIONS

PhD (Mineral Processing) Imperial College (London, England) 1976 -1979.

BSc (Pharmaceutical Chemistry) University of Chile (Chile) 1967 – 1972

CAREER DETAILS

Sustainability Division Sydney Water (previously with SWC's Australian Water Technologies) 1996-todate

Project Manager

Duties: Identify/develop technology opportunities that can lead to technology commercialisation or can be introduced to the water industry. Develop, direct and manage projects with internal and external clients. Identify, recommend areas that need consultancy investigations and R&D, organise team to carry out the projects.

THE UNIVERSITY OF NEW SOUTH WALES September 1993 - June 1996
Department of Water Engineering (Centre for Wastewater Treatment)

Program Manager Physico-Chemical Processes (also Adjunct Senior Lecturer)

Duties: Develop, lead, manage and resource R&D projects with granting organisations such as CRC Waste Management and Pollution Control, Landcare etc.

BP RESEARCH, SUNBURY-ON-THAMES, ENGLAND 1984 - Dec 1992

Senior Chemist, Environmental Engineering Team

Duties: Develop/Assess and introduce emerging environmental technologies for various business units in order to mitigate their environmental costs. Identify technology providers (European universities and companies) and negotiate both technologies testing and technology transfer.

Technologist, Mineral Processing Branch 1984 – 1990

Duties: Design, develop and assess novel technologies for introduction into the BP Minerals business in order to treat difficult ores more efficiently that with conventional technologies.

CSIRO DIVISION OF MINERAL CHEMISTRY, AUSTRALIA 1980 - 1984

Research Scientist

UNIVERSITY OF CONCEPCION, CHILE 1973-1976

Lecturer

PROFESSIONAL MEMBERSHIPS

Member of The Institute of Materials, Minerals and Mining (England)
Chartered Engineer (UK Engineering Council) Registration No 380060

MEMBERSHIP OF COMMITTEES

BP's representative at the Atomic Energy Authority / Water Research centre's Effluent Processing Club (EPC).
Member of the Working Group of Chemical Process Panel in EPC (Between Jan 1991-Dec 1992).

PUBLICATIONS

Surface Chemistry of Minerals

1. **Bustamante H**, Avella J and Castro S, Effect of Sodium Sulphide on the Flotation of Covellite, Proceedings of the III Encontro Nacional de Tratamento de Minerios (Brasil), 1975, pp 129-138.

2. **Bustamante H** and Castro S, Activating Effect of Ethylenediamine on Chrysocolla Flotation, Advances in Flotation, Univ of Concepcion, 1975, 2, 66-77 (In Spanish).

3. **Bustamante H** and Castro S, Hydrophobic Effects of Sodium Sulphide on Malachite Flotation, Trans Inst Min Metall, 1975, 84, C167-C172.

4. **Bustamante H** and Castro S, Selective Depression of Chalcocite During Molybdenite Flotation, Anais IV Encontro Nacional de Tratamento de Minerios (Brasil), 1976, pp 45-52.

5. **Bustamante H**, Sparrow G J and Warren L J, The Relevance of Model Systems in Surface in Surface Chemistry and Mineral Processing, Chemistry in Australia, 1981, 48(10), 375-379.

6. **Bustamante H** and Warren L J, Relation Between the Relative Density of Composite Coaly Grains and Their Flotation Recovery, Intern J Miner Process, 1983, 10, 95-111.

7. **Bustamante H** and Warren L J, Coal Flotation: On the Effect of Grain Size on Recovery, Proceedings of the Meeting of the Southern Hemisphere on Mineral Technology (Brasil), 1982, 1, 1-8.

8. Bennet A J R, **Bustamante H**, Telfer A and Warren L J, The Floatability of Vitrinite, Inertinite and Composite Grains in Coals of Different Ranks, Proceedings 2nd Australian Coal Preparation Conference, 1983, 161-174.

9. **Bustamante H** and Shergold H L, The Surface Chemistry and Flotation of Zinc Oxide Minerals I. Flotation with Dodecylamine, Trans Inst Min Metall, 1983, 92, C201-C208.

10. **Bustamante H** and Shergold H L, The Surface Chemistry and Flotation of Zinc Oxide Minerals II. Flotation with Chelating Agents, Trans Inst Min Metall, 1983, 92, C208-C215.

11. **Bustamante H** and Warren J L, Factors Influencing the Floatability of Australian Bituminous Coals, XV International Mineral Processing Congress (France), 1985, 2, pp 232-243.

12. **Bustamante H** and Woods G, Interaction of Dodecylamine and Dodecylsulphate with a Low Rank Bituminous Coal, Colloids and Surfaces, 1984, 12, 381-389.

13. **Bustamante H** and Rutter P R, Flocculation of Heterodisperse Suspensions of Coal, Chem Eng Sci, 1987, 43(4), 809-821.

Water/Wastewater Treatment Related Papers

14. Waite T D, **Bustamante H**, Anderson N and Brungs M, Investigations into Management Options fro Water Treatment Plant Residuals in Australia, The Management of Water and Wastewater Solids for the 21st Century, WEF Specialty Conference Series Proceedings, pages 4/49–4/60, June 19-22, 1994, Washington (USA).

15. Waite T D and **Bustamante H**, Developments in Management of Water and Wastewater Treatment Plants Sludges: Sludge Characterisation. Dewatering and Reuse, Proceedings of International Conference on Asian Water Technology 94, 311-322, (November 22-24 1994), Singapore

16. **Bustamante H** and Waite T D, New Possibilities for Dewatering and Recycle of Water Treatment Plants Residuals, Proceedings of CSIRO-UNIDO International Workshop on Modern Techniques in Water and Wastewater Treatment, (Eds L O Kolarik and A J Priestley, CSIRO Publishing, Melbourne) 163-169, 1995,

17. **Bustamante H**, Lo B and Waite T D, Conditioning and Dewatering of Water Treatment Plant Sludges, 16th AWWA Federal Convention, Volume 2, 635-641, April 1995, Sydney, Australia.

18. **Bustamante H**, Lockhart N C and Veal C J, Electrodewatering of Water Treatment Plant Alum Sludges, 16th AWWA Federal Convention, Volume 1, 869-875, April 1995, Sydney, Australia.

19. Ng K, Amal R, Raper J A, **Bustamante H** and Waite T D, Effects of Fulvic Acid on Flocculation and Dewaterability of Aluminium Hydroxide Flocs, 16th AWWA Federal Convention, Volume 2, 541-547, April 1995, Sydney, Australia.

20. **Bustamante H** and Waite T D, Innovative Techniques for the Handling and Reuse of Water Treatment Plant Sludges. Proceedings Water Osaka '95, Volume 2, 157-162, May 1995, Osaka (Japan).

21. **Bustamante H**, Emerging Technologies for the Abatement of Organic Pollutants in Industrial Effluents. 3rd National Hazardous & Solid Waste Convention. Sydney 26-30 May 1996.

22. **Bustamante H** Removal of Flotation Collectors from Wastewater, Proceedings of 2nd Conference on Cleaner Technologies for the Mineral Industry, Santiago (Chile), May 1996, 30-36.

23. **Bustamante H** and Nunez-McNally T, Low Temperature Catalytic Oxidation of VOC's: Pilot Plant Studies, Environmental Technology, vol 17, 1253-1260, 1996.

24. **Bustamante H**, C Veal and Kuo E, Stabilisation and Dewatering of Aerobic and BNR Sludges. Asian Industrial Technology Congress, 176-179, 6-8 January 1997 (Hong Kong).

25. Lo B, **Bustamante H** and Waite T D, Strength and Structure of Ferric Flocs in Water Treatment, Proceedings 17th AWWA Federal Convention, vol 1, 590-597, March 1997 (Melbourne)

26. Waite T D, Lo B, **Bustamante H** and Nguyen H, Comparison of Ferric Coagulants in Contact Filtration Pilot Plant Studies, Proceedings 17th AWWA Federal Convention, vol 1, 380-386, March 1997 (Melbourne)

27. Karaman M E, Pashley R M, Waite T D, Hatch S J and **Bustamante H**, A Comparison of the Interaction Between Model Alumina Surfaces and Their Colloidal Properties, Colloids and Surfaces A, Physicochemical and Engineering Aspects, 129/130, vol 97, 239-255, 1997.

28. Shanker S R and **Bustamante H**, *Cryptosporidium* in Perspective, Aquatic and Recreation Institute, Proceedings of Annual Conference and Trade Show, July 18-22, 1998, Penrith (Australia).

29. Karaman M E, Pashley R M, **Bustamante H** and Shanker S R, Microelectrophoresis of *Cryptosporidium* parvum oocysts in Aqueous Solutions of Various Inorganic and Surfactant Cations, Colloids and Surfaces A, vol 146, 1-3, 212–221, 1999.

30. Guan J, Waite T D, Amal R, **Bustamante H** and Wukash R, Rapid Determination of Fractal Structure of Bacterial Assemblages in Wastewater Treatment: Implications to Process Optimisation, Water Science and Technology, 38(2), 9-15, 1999.

31. Shanker R, **Bustamante H A**, Karaman M E and Pashley R M, Relevance of the Surface Chemistry of Cryptosporidium Oocysts to water Treatment, Proceedings Xth World Water Congress (CD Format) held in Melbourne in March 2000.

32. **Bustamante H A**, Shanker R, Pashley R M and Karaman M E, Interaction Between *Cryptosporidium* Oocysts and Water Treatment Coagulants, Water Research, vol 35 (13), 3179-3189, 2001

33. Cox P, Fisher I, Kastl G, Jeghatheesan V, Warnecke M, Angles M, **Bustamante H**, Chiffings T and Hawkins PT (2003) Sydney 1998-Lessons from a Drinking Water Crisis, Journal of American Water Works Association, vol 95(5), 147-161.

34. Cox P, Fisher I, Kastl G, Jeghatheesan V, Warnecke M, Angles M, **Bustamante H**, Chiffings T and Hawkins PT (2003) Sydney 1998-Lessons from a Drinking Water Crisis, Journal of American Water Works Association, vol 95(5), 147-161.

35. 2.Keegan A R, Monis P T, Daminato D, Cox P, **Bustamante H** and Saint C P (2004) Environmental and Water Treatment Processes that Contribute to Microbial Destruction - Hidden Sources of Disinfection, Water (February Issue), 29-34.

APPENDIX B

PATENT
ATTORNEY DOCKET: 63213.000003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	:	09/646,347	Confirmation No.: 8741
Applicant	:	Marilyn E. KARAMAN, *et al.*	
Filed	:	January 4, 2001	
Title	:	METHOD OF WATER PURIFICATION	
TC/Art Unit	:	1724	
Examiner:	:	Ivars C. CINTINS	
Docket No.	:	63213.000003	
Customer No.	:	21967	

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Heriberto Alejandro Bustamante under 37 C.F.R. §1.132

Sir:

I, Heriberto Alejandro Bustamante, do hereby state, that:

1. I reside at 1 Barjadda Avenue, Sylvania, NSW 2224 Australia. I received a PhD degree in Mineral Processing from Imperial College (London, England). My professional experience is detailed on my resume, attached hereto.

2. I am one of the inventors of the subject matter of US Patent Application No. 09/646347, the "present application" filed in the United States of America on 4 January 2001.

3. The present application relates to a method for the removal of biological species such as *Cryptosporidium* oocysts from water using aluminum based media which contains surface Al-OH groups.

4. According to the specification of the present application at page 5 lines 5-12, "Particulate alumina, such as powdered and granulated forms, provide an increased surface area per volume.

Powdered and granular alumina is readily available in different size ranges. Another particulate size range is from about 1.5mm to about 0.5mm. Yet another particle size contemplated by the present invention is from about 0.5mm to about 0.05mm".

5. Batch tests and pilot plant tests (details of which are provided below) were carried out for three different alumina size fractions by the Applicant. Batch tests were performed to determine the effect of particle size on the removal of *Cryptosporidium* oocysts by hydrated alumina (in bed filter material having different depths).

6. The Applicant found that an aluminum based medium which contains surface Al-OH groups, e.g. hydrated alumina (Al_2O_3), was most effective for removing *Cryptosporidium* oocysts from water if the particle size is below 1mm. The Applicant found that hydrated alumina (Al_2O_3) having a medium particle size of less than 1.0mm and more than 0.5mm was the most efficient for use in the pilot plant tests.

MATERIALS

Alumina Particle Size Fractions

7. Particle sizes of alumina assessed:

1. Large particle size of alumina (LPSA) – This size faction comprised particles that were smaller than 4mm and larger than 2mm.
2. Medium particle size of alumina (MPSA) – This size fraction comprised particles that were smaller than 1mm and larger than 0.5mm.
3. Fine particle size of alumina (FPSA) – This size fraction comprised particles that were smaller than 0.2 mm and larger than 0.1mm.

Water

8. Sydney's tap water was used in all experiments. The tap water was spikes with various amounts of gamma-inactivated *Cryptosporidium* oocysts.

Sand

9. Sand was used as control (blank) filter material.

METHODS

Cryptosporidium Oocysts Removal Tests

10. Two types of removal tests were assessed in the study, namely, batch tests and continuous tests. The batch tests were designed to identify a particle size that would be most effective for use in the pilot plant test. The pilot plant tests were designed to maximize the removal of *Cryptosporidium* oocysts from a substantial volume of water.

Batch Tests

Procedure to run batch tests.

11. Batch tests were carried out in a glass column (28mm x 18cm). The glass column had a 90 micron sintered filter at the bottom to hold the particles. Before each batch test the glass column was thoroughly rinsed with tap water. In addition, the column was further cleaned in an ultrasound bath after every third experiment.

12. The glass column was loaded with hydrated alumina particles of fine, medium, or large size. The amount of hydrated alumina particles in the glass column differed depending on the particle size being tested. The loaded column was filled with water to around 2cm above the hydrated alumina particles to prevent working with a "dry" bed of particles.

13. A 5µL aliquot containing 10^5 oocysts/mL was added to 30mL of tap water to obtain 500 oocysts/30mL. The dispersion was vortexed for around 30 seconds to ensure maximum homogenization and promote de-clumping of the oocysts.

14. All 30mL of tap water containing 500 *Cryptosporidium* oocysts was added to the glass column for one minute after which the water was allowed to flow out. The typical filtration time from the bed of particles was about 2 minutes. After the water exited the column, the bed of hydrated alumina particles was rinsed with 3 portions of 5 mL of *Cryptosporidium* free tap

water. This procedure was designed to ensure that any "loose" *Cryptosporidium* oocysts that may be mechanically trapped in the column were released.

15. All of the rinsing water was collected and combined with the initial 30mL. The total volume of water was approximately 200mL. The number of *Cryptosporidium* oocysts was determined in each 200mL water sample. This procedure was adopted to avoid subsampling of the filtered water and minimise analytical error. Sand (or in some cases glass spheres) was used as a control material (i.e., a blank) for each of the various particle sizes of hydrated alumina.

Continuous Tests

16. Continuous tests were carried out in a small pilot plant. The pilot plant comprised (i) a 400 L PVC tank, (ii) a variable flow pump and (iii) a glass column (50cm x 4cm) comprising medium size alumina particles (MSPA) or sand. The height of the MSPA or sand bed in the column was about 32cm.

Pilot plant operation

17. The glass column was prepared by placing a 90 micron sintered filter at the bottom and then loading the column to a bed height of about 32 cm with MSPA or sand. Water was added to the column to about 5cm above the level of the bed of particles. The 5cm level of water above the bed of particles was maintained constant to avoid working with a "dry " bed.

18. *Cryptosporidium* oocysts were added to the 400L PVC tank with tap water. The water in the tank was continuously stirred to minimize settling of the *Cryptosporidium* oocysts. The *Cryptosporidium* oocysts and water were then pumped to the glass column.

19. The average number of *Cryptosporidium* oocysts in the water fed to the glass column was approximately 3,000 – 3,500 per litre. Under these conditions it was possible to run the pilot plant for 20 days.

20. Sand was used as the control (blank) filter material for the pilot plant tests. The particle size range of the sand used as control was similar to that of MSPA, namely the sand particles were smaller than 1 mm and larger than 0.5mm.

Results for batch tests

21. Table 1 shows the effect of particle size and depth of the filter bed on the removal of *Cryptosporidium*:

TABLE 1

Particle Size Fraction	Depth of particle bed (mm)	Oocyst Removal (%)
Fine particles (less than 200µm more than 100µm)		
	3	95
	5	96
	10	98
Medium size particles (less than 1.0mm more than 0.5mm)		
	50	82
	100	89
	150	96
Large size particles (less than 4mm more than 2mm)		
	50	21
	100	41
	150	58

22. Thus, it is apparent, with particles having sizes in the range of about 1mm or less, oocyst removal of more than 80% and even up to 98% can be reached. For particles larger than 2mm,

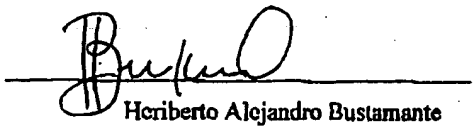
the percentage removal is much smaller. Particles smaller than 200µm showed good oocyst removal, however fine particles are not suitable for application in the pilot plant because the filtration rate is low and would require a pressurized filtration system rather than gravity alone.

Results for pilot plant operation

23. The operation of the pilot plant demonstrated that particles having sizes in a range of about less than 1mm and more than 0.5mm are workable in a gravity fed filtration system, and enable a reasonable reduction in oocysts. Tests carried out using large particle size alumina particles resulted in less than 10% removal of *Cryptosporidium* oocysts.

24. The average number of oocysts in the column of medium sized alumina particles (less than 1.0mm and more than 0.5mm) was approximately 3,000 - 3,500 oocysts/l. Under these conditions it was possible to continuously treat the oocyst-containing water for 20 days. Over the 20 days the column was fed with around 9,000,000 oocysts in total. During this time the oocysts removal by the bed of medium size alumina particles was consistently between 2.5 and 3.5 log removal. By comparison the removal of oocysts by medium size sand particles only reached 0.6 log removal in a period of one week and its use was therefore discontinued.

25. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed 

Heriberto Alejandro Bustamante

Place Sydney (Australia)

Date 7 February 2006

APPENDIX C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	:	09/616,347
Applicant	:	Marilyn E. KARAMAN, *et al.*
Filed	:	January 4, 2001
Title	:	METHOD OF WATER PURIFICATION
TC/Art Unit	:	1724

Declaration of Heriberto Alejandro Bustamante under 37 C.F.R §1.132

Sir:

I, Heriberto Alejandro Bustamante, do hereby state that:

1. I reside at 1 Barjadda Avenue, Sylvania, NSW 2224 Australia. I received a PhD degree in Mineral Processing from Imperial College (London, England). My professional experience is detailed in my resume, previously provided to you with my Declaration of 7 February 2006, filed 8 March 2006.

2. I am one of the inventors of the subject matter of US Patent Application No. 09/646,347, the "present application" filed in the United States of America on 4 January 2001.

3. As stated in my previous Declaration, batch tests and pilot plant tests were carried out for three different alumina size fractions by the Applicant. Batch tests were performed to determine the effect of particle size on the removal of Cryptosporidium oocysts by hydrated alumina. The Applicant found that hydrated alumina (Al_2O_3) having a medium particle size (i.e less than 1.0mm and more than 0.5mm) was the most efficient for use in the pilot plant tests.

4. The Examiner alleges that the results of the present invention are not unexpected since a volume of particles having a small diameter would produce more surface area than the same volume of particles having a larger diameter.

5. However, the difference in the efficacy of adsorption seen in particles of different diameters is not attributable to the change in surface area *per se*.

6. Table 1 (below) provides a comparison between geometric surface area (cm^2/g) available for various particle sizes of alumina particles and area occupied by $1x10^6$ *Cryptosporidium* oocysts.

7. Referring to Table 1, a person skilled in the art, and with knowledge of surface area chemistry, would estimate that the geometric surface area of a large spherical alumina particle having a diameter of 0.4cm is approximately 3.8 cm^2 per gram. The total area occupied by 1 million oocysts would be 0.2cm^2. Based on adsorption studies on flat alumina surfaces, it is estimated that an alumina particle surface area of about 100 times the area occupied by a *Cryptosporidium* oocysts would required to capture one oocyst. That is, a surface area of about 2.0E-5 cm^2 would be required to adsorb each oocyst. This means for example that 4g of particles having a diameter of 0.3-0.4cm would be sufficiently large to adsorb 1 million *Cyrptosporidium* oocysts.

Alumina Size, cm	Alumina mass, g	Alumina density g/cm3	Volume of one alumina particle, cm3	Volume of ONE GRAM of alumina, cm3	Area of ONE alumina particle, cm2/particle	Number of alumina particles in 1 g of alumina	Area of 1 g of alumina, cm2	Oocyst diameter, cm	Cross sectional area of one oocyst, cm2	Area occupied by 1 million oocysts, cm2
0.1	1	3.97	5.23E-04	2.52E-01	3.1E-02	4.8E+02	15.1	5.00E-04	1.96E-07	1.96E-01
0.005	1	3.97	6.54E-08	2.52E-01	7.9E-05	3.9E+06	302.3	5.00E-04	1.96E-07	1.96E-01
0.3	1	3.97	1.41E-02	2.52E-01	2.8E-01	1.8E+01	5.0	5.00E-04	1.96E-07	1.96E-01
0.2	1	3.97	4.19E-03	2.52E-01	1.3E-01	6.0E+01	7.6	5.00E-04	1.96E-07	1.96E-01
0.4	1	3.97	3.35E-02	2.52E-01	5.0E-01	7.5E+00	3.8	5.00E-04	1.96E-07	1.96E-01

Table 1

Pilot Plant Operation

8. As described in my previous Declaration at paragraph 17, we conducted a Pilot Plant Operation wherein a glass column was prepared by placing a 90 micron sintered filter at the bottom and then loading the column to a bed height of about 32cm with alumina particles. The loaded columns hold about 100g of alumina particles.

9. As explained in paragraph 24 of my previous declaration, around 9 million oocysts were fed through the column over 20 days.

10. Referring then to the calculations of Table 1 and paragraph 7, if a column is packed with 100g of particles having a diameter of about 0.4cm the total surface area provided is enough to adsorb more than 20 million oocysts.

11. In fact, the larger particle sizes tested in the Pilot Plant Operation had an average particle size of about 0.25-0.3cm. Accordingly the total surface area available for adsorption of oocysts using the large particle size in the Pilot Plant Operation would have been about 600cm^2 and would be enough to adsorb *more than* 20 million oocysts.

12. Accordingly, in theory the larger particle sizes would provide more than enough surface area to adsorb all of the oocysts fed through the column in the 20 day period of the Pilot Plant Study.

13. The larger particles were considered to be particularly useful in the Pilot Plant Study as they can be used under in a gravity fed system. By contrast, use of smaller particles in the columns of the Pilot Plant Study are expected to cause a pressure drop and a decrease in throughput such that commercial use of smaller particles would require a pressurized filtration system to maintain throughput.

14. Also, larger particles are easier to handle during cleaning of the column. The cleaning process requires that the particles are back washed by forcing air through the column from the bottom to the top to form a fluidized bed system. The larger particles are heavy enough to be retained in the column whereas smaller particles are often lost in this process as they are expelled from the top of the column.

15. However, as evidenced in Table 1 of my previous Declaration and as stated in paragraph 22, particles larger than 2mm removed only a small percentage of oocysts in the batch tests. Furthermore, as explained in paragraph 23 of my previous Declaration, Pilot Plant studies carried out using the large particle size alumina particles resulted in less than 10% removal of *Cryptosporidium* oocysts. Accordingly even though the larger particle sizes theoretically provided more than sufficient surface area to adsorb the *Cryptosporidium* oocysts, the larger particles were not effective.

16. The present invention was achieved by selecting a particle size that is only slightly smaller than the large particle size. The oocyst removal by the bed of medium sized alumina particles (1.0mm-0.5mm) was consistently between 2.5 and 3.5 log removal. In view of the calculations provided above the improved adsorption cannot be attributed to the increase in surface area provided by the decrease in particle size *per se*.

Signed _____

Heriberto Alejandro Bustamante

Place Sydney, Australia

Date 28 September 2006

APPENDIX D

IN THE UNITED STATES PATENTS AND TRADE MARK OFFICE

Applicant: The Australian National University and Australian Water Technologies Pty Ltd
Serial Number: 09/646347
Filed: 18 March 1999
Title: Method of Water Purification
Examiner Ivars C. Cintins

DECLARATION UNDER Rule 132

I, Marilyn Karaman of 105 Bandjalong Crescent, Aranda, ACT, Australia, declare that:

1. I am one of the inventors of the subject matter of US Patent Serial No. 09/646347 (hereinafter referred to as "the present application") filed on 18 March 1999.

2. My qualifications and technical experience are set out in my *Curriculum vitae*, a copy of which is attached as Annex A.

3. My *Curriculum vitae* demonstrates that I have substantial experience in the art of water purification and particularly the removal of the protozoa from water.

4. The invention of the present application results from a study of the flocculation process for a variety of water treatment chemicals and their interaction with environmental pathogens such as *Cryptosporidium parvuum* (hereinafter referred to as *Cryptosporidium*).

5. Looking at *Cryptosporidium* particularly, we tested a range of inorganic solids including fluorspar, geothite, rutile, pyrite and silica along with hydroxylated alumina (CryptoBlast™) to measure each of their ability to adsorb oocysts of *Cryptosporidium in vitro*. The results of this experiment are shown in Figure 1, attached herewith as Annex B.

6. With the exception of hydroxylated alumina, the results indicate that oocysts of *Cryptosporidium* readily pass through columns filled with each of the inorganic solids tested. The experiment showed that, of all the solids tested, the oocysts strongly adsorbed onto the hydroxylated alumina only.

7. The next question was whether the alumina must be hydroxylated to have its effect on adsorbing oocysts of protozoa or whether unhydroxylated alumina would have the same efficacy. In this regard, we carried out a series of experiments using 200μm unhydroxylated alumina particles.

The unhydroxylated alumina was prepared by heating hydroxylated alumina at 610°C in the presence of air for two and a half hours such that the hydroxyl groups were removed.

8. Around 1 gram of 200μm unhydroxylated alumina was tested in a column with a dispersion of *Cryptosporidium* in water, the results of which are shown in Figure 2, attached herewith as Annex C.

9. We found that, upon addition of the *Cryptosporidium* containing water to the column, around 10% of the *Cryptosporidium* would be immediately released (permeate in Figure 2). Furthermore, further washing of the column with water removed the remaining *Cryptosporidium*. Thus, washing the column for the fifth time with water resulted in release of almost 75% of the *Cryptosporidium* from the column.

10. As indicated in Figure 2, we observed the complete opposite behaviour for hydroxylated alumina. In this case, the *Cryptosporidium* were irreversibly adsorbed on the hydroxylated alumina and no *Cryptosporidium* were released by washing the column with water.

11. I have undertaken a review of US Patent No. 6,054,059 by Latimer *et al* (hereinafter referred to as the "Latimer patent").

12. The Latimer patent describes a filtration material which includes mineral substrate coated with various metal oxides such as Al_2O_3, MgO or SiO_2. The "coating" of metal oxide is achieved by heating mixtures of the mineral substrate and the metal oxides at temperatures between 2000 and 2200 °F.

13. Under these temperature conditions, the metal oxides Al_2O_3 (and SiO_2) will lose their surface hydroxyl groups giving rise to the formation of unhydroxylated, hydrophobic coatings on the mineral substrate.

14. The Latimer patent would appear to rely upon electrical affinity between the metal oxides and the protozoa as discussed at column 8 line 64 to 67:

"In addition to providing the filtration material with a desired electrical affinity, the surface metal oxides serve as parting agents to prevent the prills from sticking together as the intense heat is applied during firing."

It is unclear from a reading of this document as to how the alumina in an unhydroxylated form can act in this manner to remove protozoa from water.

Further, the surface of the agglomerated fine minerals would not be completely coated with the unhydroxylated alumina. In this regard, I refer to column 9, lines 3-9 of the Latimer patent:

"During firing, some of the surface may be occupied by the surface metal oxide and other portions of the surface may be occupied by the mineral fines. If magnesium oxide is used, a higher percentage of magnesium oxide on the surface may be required compared to the amount of aluminium oxide on the surface"

15. The hydroxylated alumina of our invention does not rely upon electrostatic charges as suggested by the Latimer patent. Rather, the present invention relies on the strong, specific chemical interaction between hydroxylated alumina and carboxylic groups present on the surface of protozoa. Furthermore, our experimental work shows that the adsorption of protozoa on the hydroxylated alumina is independent of the charge of the alumina and adsorption takes place even when both the hydroxylated alumina and the protozoa carry the same charge. The experimental work in question is

described in the specification of the present application at page 14, line 28 to page 15, line 10.

16. The alumina described in the Latimer patent does not have the properties of the hydroxylated alumina of the present invention. In this regard, the Latimer patent teaches the use of unhydroxylated alumina which we have shown experimentally to be ineffective in adsorbing protozoa oocysts.

The undersigned declarant declares that all the statements made herein of her own knowledge are true and that all statements made as information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both under the United States Code and that such wilful false statements may jeopardise the validity of the application or patent issuing thereon.

Dated this 27th March 2002

M. K

MARILYN KARAMAN

Applicant: The Australian National University and Australian Water Technologies Pty Ltd
Serial Number: 09/646347
Filed: 18 March 1999
Title: Method of Water Purification
Examiner Ivars C. Cintins

ANNEX A

The attached is Annex A referred to in the Declaration made by Marilyn Karaman on 27/3/02 2002

M. Ic

MARILYN KARAMAN

Curriculum Vitae

NAME: **Marilyn E. Karaman**
ADDRESS: 105 Bandjalong Cres., Aranda, ACT 2614
NATIONALITY: Australian
PROFESSION: Analytical and Physical (Surface) Chemist

EDUCATION/QUALIFICATIONS :

Chemistry Certificate , Sydney Technical College, (Completed in 1978)

Associate Diploma in Applied Science (Chemistry), Canberra College of Advanced Education (Completed in 1984).

Degree of Bachelor of Applied Science (Chemistry), Canberra College of Advanced Education (Completed in 1985).

Graduate Diploma in Science Pass with Merit (Chemistry), The Australian National University (Completed in 1991).

Graduate Teaching Program Certificate, The Australian National University, (Completed in 1995)

Degree of Doctor of Philosophy (Chemistry), The Australian National University (Awarded September 2000).

SUMMARY OF PhD EXAMINERS COMMENTS
Thesis entitled: "Experimental and Theoretical Studies on Surfactant Wetting and Surface Forces".
Examiners : Prof. Jim Quirk (University of Western Australia), Prof. Tom Healy (Melbourne University) and Dr Tommy Nylander (Sweden).

Examiner (1)

Stated that each chapter in the thesis was "cutting edge" in the designated topic, "self contained". "The thesis is truly a substantial contribution". "The candidate has demonstrated that she is a versatile and accomplished laboratory chemist" "the most noteworthy features of this thesis is the variety of experimental skills and techniques she has used". The various chapters were described as having "excellent and comprehensive set of experiments", "quite superb experiments with all the care demanded", "fascinating results", "the thesis taken as a whole is a tour de force".

"In terms of presentation the candidate writes with clarity and economy of style which to me indicates someone thoroughly conversant with the subjects treated and with their theoretical background and connotations". "The thesis is so well illustrated that many book authors could learn from the candidates approach".

"A substantial part of the research has been already been published in first class

"Some Universities have a category cum laude and it is my opinion that Ms Karaman's thesis resides comfortably within this category. Accordingly I recommend that she should be favourably considered for a University Prize".

Examiner (2)

"It was a great pleasure to read this thesis as it covers such a broad spectrum of problems in surface and colloid science. Many of the experiments presented are really clever. The PhD candidate has used a number of techniques that by themselves are quite tricky yet she has obtained data of very good quality. The amount of work put in this thesis is impressive and more than what is usually expected for a PhD. The thesis also arises new, relevant and interesting questions, which I'm sure, will be topics for PhD theses in the future".

The various chapters were described as "impressive", "new" and "interesting" experiments "very clever and very well carried out".

Examiner (3)

"This thesis is the best produced one I have ever read : I congratulate the author" "Almost all of this thesis is a delight to read and study".

The various chapters have been described as "interesting, useful and worthwhile work" "thought provoking", "concise", "tantalising stuff" containing "some very elegant experimental work".

SUMMARY OF RELEVANT WORK EXPERIENCE:

Over the last 25 years I have gained extensive experience in laboratory practice and research in both analytical and physical chemistry. During my undergraduate studies I completed a major in Chemistry with electives in Medical Technology units. I have a strong background in Atomic Force Microscopy, TEM, SEM, organic synthesis and surface chemistry (both as a RA II and as a graduate student). I have also gained valuable experience in microbiological techniques, MLC, radioimmunoassays, radiolabelling (organic synthesis) and a feel for medical research in general, because of my involvement in immunosuppression research in the John Curtin School of Medical Research (JCSMR) at the ANU.

In addition, I have been involved in membrane design and production of a new generation of *filtration (ultra and desalination) membranes* and the design of a novel fully automated *instrument for the determination of the Minimum Film Formation Temperature of Latex*. I have recently been carrying out research involving the *removal of Cryptosporidium from water*. It should be noted that ***all three projects*** have resulted in the lodgement of patent applications naming me as an ***inventor***.

July 1974 - December 1975: Marrickville Holdings (ETA Division), *Trainee Chemist*, Analysing a wide range of food products, raw materials and in process foods.

December1975 - July 1977: A.P.D. Snack Foods, *Quality Control Analyst*, Analysing a wide range of food products,raw materials and in process foods.

July 1977 - September 1978: Soul Pattinson Laboratories, *Laboratory Analyst (Quality Assurance)*, Responsible for quality assurance analysis on a wide range of pharmaceutical products, raw materials and the in-process goods.

September 1978 - April 1980: Helena Rubinstein, *Quality Assurance Chemist*, Performing similar duties as above for a wide range of cosmetic products involving both chemical and instumental analysis as well as colour matching.

July 1980 - September 1985: Department of Chemistry, ANU, *Technical Officer*, I played a supporting role in the teaching laboratories involving the preparation of undergraduate starting materials and lecture demonstrations, preparation and standardisation of solutions required for practical classes. Recovery of precious metals. Demonstrating techniques involved in undergraduate radiochemistry experiments. Operating the AAS and 360M NMR as a service for undergraduate classes.

September 1985 - July 1997: Department of Applied Maths, Research School of Physical Sciences and Engineering, ANU, *Research Assistant Grade II*, Involving varied interdisciplinary research which in the past has involved immunology (radioimmuno -assays) - MLC, radiolabelling, organic synthesis, freeze fracture/freeze etch, TEM, SEM, membrane design using polymerisable microemulsions, imaging AFM, *Colloid Probe AFM* measuring forces in a variety of systems including between cast latex films in the presence of various additives.

August 1997 - November 1997: Department of Chemistry, The Faculties, ANU, *Research Assistant Grade II*, Measuring forces in a variety of systems using LLIFE (light lever instrument for force evaluation). LLIFE is an in-house built AFM dedictated solely for surface force measurements.

November 1997- April 1998: Australian Water Technologies - Ensight (West Ryde, Sydney), *Consultant*, Involved in interdisciplinary research, using surface chemistry techniques such as microelectrophoresis, FESEM and Atomic Force Microscopy to study the pathogenic protozoa *Cryptosporidium*.

May 1998- April 2000: Australian Water Technologies-Ensight, *Environmental Scientist*, Involved in environmental pathogens (e.g *Cryptosporidium*) and water treatment research. Standard surface chemistry techniques and AFM were used to study the flocculation process for a variety of water treatment chemicals and their interaction with environmental pathogens such as *Cryptosporidium parvuum*.

April 1998- April 2000: *Visiting Fellow* in the Department of Chemistry, The Faculties, Australian National University.

April 2000-Present: Department of Chemistry, The Faculties, ANU, Research Associate, measuring forces between fluorocarbon coated surfaces to elucidate the nature of the *Hydrophobic Interaction*.

Grants
ARC SPIRT Grant with AWT-Ensight (NSW) for 'Surface characterisation of *Cryptosporidium* oocysts for the development of novel filtration systems for commercial applications' ($140,000).

REFERENCES ARE AVAILABLE FROM:

(1) Professor Richard Pashley (Chair of The Board of The Faculties), The Australian National University, Email: richard.pashley@anu.edu.au.
Ph: 61-02 6249 2631.

(2) Professor Barry Ninham (Head of Department), Dept. of Applied Maths, Research School of Physical Sciences and Engineering, The Australian National University, Email: barry.ninham@anu.edu.au., Ph: 61-02 62492470.

(3) Professor J.P.Quirk, School of Agriculture, University of Western Australia, Nedlands WA 6009, Ph: 61- 08-93802769, Fax: 61-08-93801050.

(4) Professor Tom Healy (Head of Department), School of Chemistry, University of Melbourne, E-mail: t.healy@chemistry.unimelb.edu.au., Ph: 61-3-93446481.
fax: 61-03-93446233

REFEREED INTERNATIONAL JOURNAL ARTICLES AND PATENTS

(1) M.E.Karaman, L.Meagher and R.M.Pashley. Surface Chemistry of Emulsion Polymerisation, *Langmuir*, **9**, 1220-1227 (1993).

(2) M.E.Karaman, B.W.Ninham and R.M.Pashley. Some Aqueous Solution and Surface Properties of Dialkyl Sulfosuccinate Surfactants, *The Journal of Physical Chemistry*, **98**, 11512-11518 (1994).

(3) M.E.Karaman, R.M.Pashley and N.K.Bolonkin. Study of the Surface and Biological Activity of a Trivalent Cage Surfactant., *Langmuir*, **11**, 2872-2880 (1995).

(4) R.M. Pashley, B.W. Ninham, S.T. Hyde, M.E. Karaman and R.A. Morris
'Formation of porous materials.' US Patent No 5,529,690, issued 25 June 1996.
(5) M.E.Karaman, B.W.Ninham and R.M.Pashley. Effects of Dissolved Gas on Emulsions, Emulsion Polymerisation, and Surfactant Aggregation, *The Journal of Physical Chemistry* , **100**, 15503-15507 (1996).

(6) R.M.Pashley, M.E.Karaman, B.W.Ninham., Method and Apparatus for the Measurement of Film Formation Temperature of a Latex., *Provisional Patent Submitted* (February 1997).

(7) M.E.Karaman, R.M.Pashley, T.D.Waite, S.J.Hatch and H.Bustamante. A Comparison of the Interaction Forces Between Model Alumina Surfaces and Their

Colloidal Properties, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **129-130**, 239-255 (1997).

(8) V.Yaminsky, B.W.Ninham and M.E.Karaman. Dewetting of Mica Induced by Simple Organic ions Kinetic and Thermodynamic Study., *Langmuir*, **13**, 22, 5979-5990 (1997).

(9) M.E.Karaman, R.M.Pashley, H. Bustamante and S.R.Shanker., Method of Water Purification., *PCT International* (Publication date: September 1999).

(10) R.M.Pashley, M.E.Karaman, V.S.J Craig and M.M.Kohonen. Use of the Light-Lever Technique for the Measurement of Colloidal Forces., *Colloids and Surfaces A: Physicochemical and Engineering Aspects* , **144**, 1-3, 1-8 (1998)

(11) M.E.Karaman, R.M.Pashley, H.Bustamante and S.R.Shanker. Micro-electrophoresis of *Cryptosporidium* parvum Oocysts in Aqueous Solutions of Inorganic and Surfactant Cations., *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **146** n1/3, 217-226 (1999).

(12) R.M.Pashley and M.E.Karaman. The Role of the Meniscus in the Drying of Latex films, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, - In Press.

(13) M.M.Kohonen, M.E.Karaman and R.M.Pashley. Electrical Double Layer Forces in the Presence of Multivalent Electrolytes, *Langmuir* **16(13)**: 5749-5753, 2000.

(14) S.R.Shanker, M.E.Karaman, H.Bustamante and R.M.Pashley. Surface Characteristics of *Cryptosporidium* Oocysts: Implications for Pilot Plant Studies, *Journal of Environmental Microbiology* - Submitted.

(15) H.Bustamante, M.E.Karaman, S.R.Shanker and R.M.Pashley. Effect of Coagulants and Polyelectrolytes on the Surface Properties of *Cryptosporidium, Water Research*- In Press.

(16) M.E.Karaman, D.A.Antelmi and R.M.Pashley. Hydrocarbon and Fluorocarbon Carboxylic acid Adsorption onto Alumina Substrates, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, - In press.

(17)M. E. Karaman, R. M. Pashley, S.R. Shanker, and H. Bustamante
'Destruction of *Cryptosporidium* Oocysts by Adsorption onto Active Solids'
International PCT Application (Submitted March 2001).

REFEREED CONFERENCE PAPERS

S.R.Shanker, H.Bustamante,M.E.Karaman and R.M.Pashley. Relevance of the Surface Chemistry of Cryptosporidium Oocysts to Water Treatment Plants. Proceedings of the Xth World Water Congress, Melbourne Australia, February 2000

Applicant: The Australian National University and Australian Water Technologies Pty Ltd
Serial Number: 09/646347
Filed: 18 March 1999
Title: Method of Water Purification
Examiner Ivars C. Cintins

ANNEX B

The attached is Annex B referred to in the Declaration made by Marilyn Karaman on 27/3/02 2002

M. K

MARILYN KARAMAN

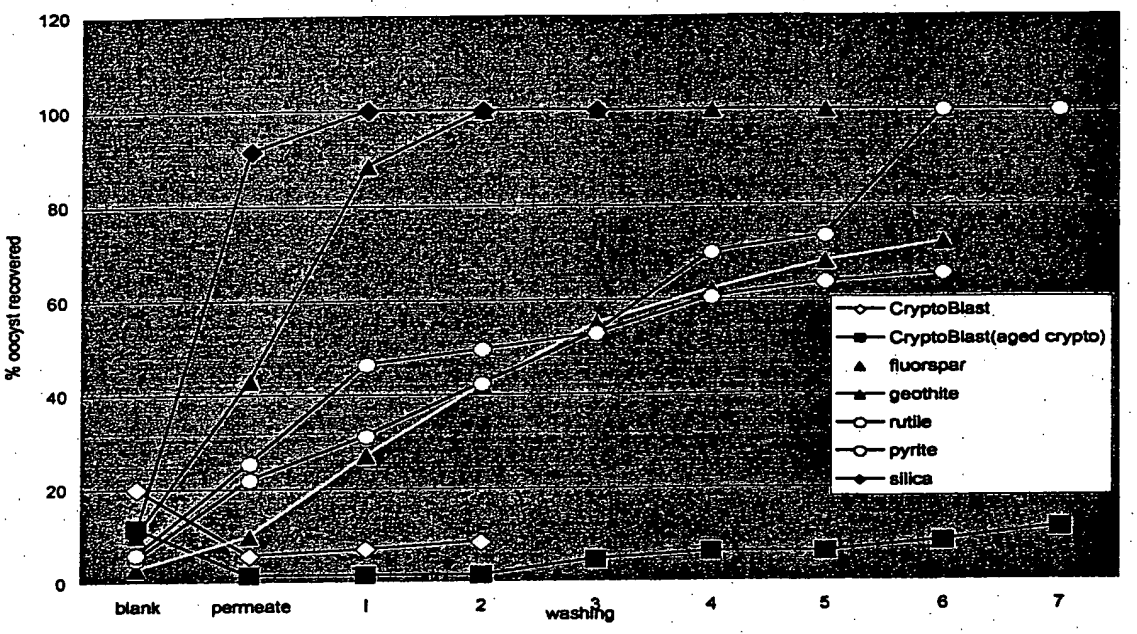


Figure 1. Comparison between hydroxylated alumina (CryptoBlast™) and a wide range of other inorganic solids on oocysts elution from column with water.

Applicant: The Australian National University and Australian Water Technologies Pty Ltd
Serial Number: 09/646347
Filed: 18 March 1999
Title: Method of Water Purification
Examiner Ivars C. Cintins

ANNEX C

The attached is Annex C referred to in the Declaration made by Marilyn Karaman on 27/3/02 2002

M. K

MARILYN KARAMAN

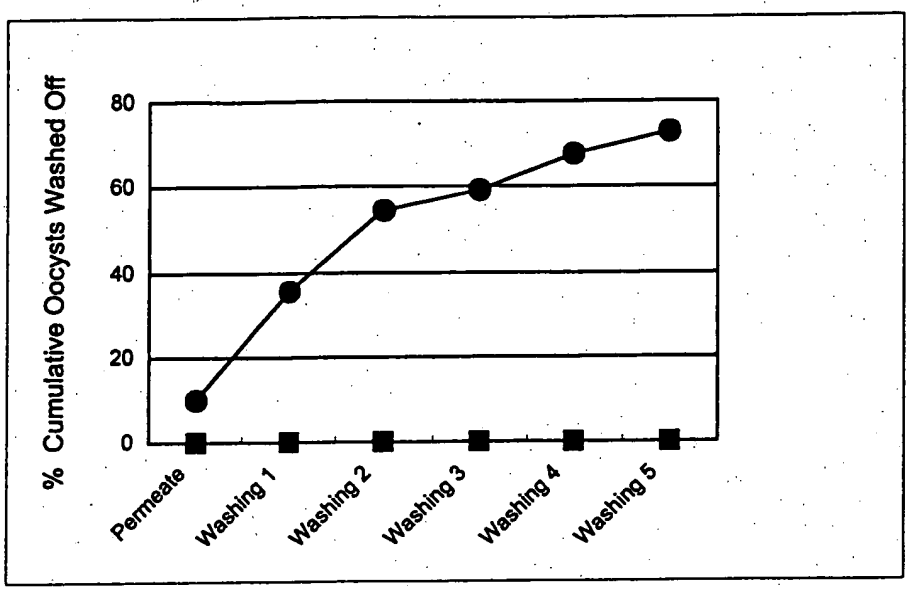


Figure 2. *Cryptosporidium* on unhydroxylated alumina (n) are readily removed by washing with water as opposed to *Cryptosporidium* on hydroxylated alumina (g) onto which the oocysts are irreversibly adsorbed

APPENDIX E

The Determination of the "True" Filtration Characteristics of Diatomaceous Earth[1]

Robert J. R. Reed and Mark A. Picksley, *Brewing Research Foundation, Nutfield, Redhill, Surrey, England RH1 4HY*

ABSTRACT

The flow through any filter bed is directly related to the permeability of the bed, which may be calculated from the size of particles comprising the bed and the void volume (or voidage) between the particles. Conventional measurement by Coulter Counter of the particle size of diatomaceous earth and void volume by displacement of volume leads to unrealistically high values of permeability. This is because the influence of the porosity (internal void volume) of the diatomaceous earth particles is not accounted for in the straight application of conventional measurements to the calculation of permeability. A method was developed whereby the particle size and bed void volume relevant to filtration may be determined by the analysis of data on the volumes of beds comprising mixtures of diatomaceous earths and particles of known filtration characteristics. The diatomaceous earths Celite 578, Standard Supercel, and Hyflo Supercel have total void volumes of 83, 86, and 86%, respectively; the effective bed void volumes for filtration, however, are only 27, 33, and 58%. These results imply that true sizes of diatomaceous earth particles are some 1.7 times greater than indicated by the Coulter Counter. The method also gives an indication of the size of the voids in a bed of filter aid. Effective bed void volume and true particle size give reasonable predictions of permeability.

Key words: Diatomaceous earth, Filtration, Filter-bed permeability, Void volume, Particle size, Particle shape

It seems remarkable that information on diatomaceous earths is insufficient to allow a brewery filter room manager to select the type and quantity of filter aid for any given task from data sheets alone, without recourse to laboratory experiments and experience on full-scale plant. Although suppliers' data for diatomaceous earth are available, most of the information is not that required by the filter room manager and may even be misleading for filtration decisions. To assist in the selection of the type and quantity of filter aid, the manager needs to know the permeability of the filter aid, the proportion of the filter cake that is available for the entrapment of beer solids, and the sizes of the voids and other pores that determine the particle cutoff size in the filter.

Filtration of beer using diatomaceous earth as bodyfeed follows the laws of filtration as defined by the Darcy or pressure equation (1).

$$\Delta P = \frac{\mu u L}{\beta} \qquad (1)$$

and

$$\beta = \frac{\epsilon^3}{Ks^2(1-\epsilon)^2} \qquad (2)$$

where ΔP = pressure drop across the filter; μ = viscosity of beer; u = nominal velocity or flow rate per unit area; L = depth of the filter; β = permeability of the filter cake; and ϵ = void volume or voidage of the filter cake; s = specific surface area of particles, i.e., the surface area of particles per unit volume of particles; and K = constant (Table I).

These equations can be derived from basic principles and have been shown to hold true in practice (7). The permeability frequently quoted by suppliers is derived via equation 1 from the measurement of pressure drop across a bed of filter aid. As such it is a true experimental result that, allowing for variability in the material, is not open to question.

Permeability depends on the void volume, ϵ, of the filter cake and the specific surface area of the particles making up the bed. The specific surface area, s, is primarily related to the size of the particles and to some extent the shape of the particles. For spheres it is equal to six divided by the diameter. The constant K in equation 2 also depends on the shape of the particles, and is generally between two and eight but is commonly five (1).

For a bed comprised of particles of known shape and size, it is a simple matter to calculate the specific surface area and to measure the void volume. Unfortunately, the materials used for beer filtration are not so easily characterized. They are rarely spherical. Diatomaceous earths in particular have complex structures that incorporate part of the void volume into individual particles. In fact, as can be seen in Table II, conventional measurements of void volume and particle size (assuming spherical character for calculation of specific surface area) result in calculated permeabilities (from ϵ_t and d_{CC}, Table II) far greater than the true value calculated from pressure drop data and equation 1.

Whereas an estimate of specific surface area of filter aid particles can be calculated from conventional particle sizing techniques such as Coulter counting, it is essential that the physical characteristics of the filter aid are allowed for in this calculation. Where materials that have a distribution of sizes are concerned, it is important that any mean particle diameter should reflect the specific surface area of the material. That is to say, smaller particles have a greater specific surface area than larger particles on a volume to volume basis. Accordingly, in this paper the mean diameters are calculated from the specific surface area of particles derived from Coulter Counter data. Any direct measure of specific surface area using Brunauer-Emmett-Teller (BET) adsorption techniques does not correspond to the overall size and shape of complex structures such as diatoms, but rather to surface roughness and convoluted internal structure. Thus the specific surface area available for the adsorption of gases has little relevance to filtration other than giving an indication of the permeability of the diatoms' walls.

The calculation of permeability from equation 2 is particularly sensitive to void volume, or perhaps more accurately, the effective bed void volume, i.e., the volume of the voids in the bed *between* the particles of filter aid. It is also the effective bed void volume that gives an indication of the space available in the filter aid for the

TABLE I
Definitions of Terms and Symbols

Symbol	Definition
ΔP	Pressure drop ($N \cdot m^{-2}$)
μ	Viscosity ($kg \cdot m^{-1} \cdot s^{-1}$)
u	Nominal velocity ($m \cdot s^{-1}$)
L	Depth (m)
d	Particle diameter (m)
d_{cc}	Particle diameter, measured by Coulter Counter (μm)
d_{eff}	Effective particle diameter (μm)
s	Surface area of particles per unit volume of particles (m^{-1})
ϵ	Void volume
ϵ_t	Total void volume (%)
ϵ_b	Effective bed void volume (%)
β	Permeability ($m^2 = 10^{12}\ \mu m^2$)
K	Permeability equation constant
ρ_s	Density of solid fraction of filter aid ($g \cdot ml^{-1}$)
ρ_b	Dry bulk density of bed of filter aid ($g \cdot ml^{-1}$)

[1]Presented at the 52nd Annual Meeting, Tucson, AZ, May 1986.

entrapment of beer particles. It is the measurement of this effective bed void volume, and its influence on effective particle size, which is described in this paper.

EXPERIMENTAL

Measurements of Effective Bed Void Volume—Principles

The simplest method, both in concept and operation, to assess the effective bed void volume with respect to filtration, is to determine experimentally the volume of very fine material (compared to diatomaceous earth particles) that can be introduced into the bed of diatomaceous earth without increasing the volume of the bed. Providing the particle size of the added material (filler) is very much smaller than the size of the voids in the bed, small additions should not increase bed volume. The addition of further filler will only increase the volume of the bed, as shown by line 1 in Figure 1, when the voids between the filter aid particles are full. At this point the volume of the bed will increase by the volume of filler added, that is to say, the gradient of the line will be unity. It should be noted that the volume of the filler is the total bed volume of the filler material, i.e., the wetted bulk volume.

Thus, for a diatomaceous earth with an effective bed void volume of 50%, the point of intersection of the line of unit slope and the x-axis should be equivalent to 50 ml of filler per 100 ml of earth.

Line 1 (Fig. 1) is a somewhat idealized case, although it can be approached, as will be shown later. When the particle size of the filler is not sufficiently small, there will be a tendency for the overall volume of the bed to increase prematurely (line 2) even though not all of the bed void volume between the diatomaceous earth particles is filled. Preferably, the filler comprises particles with a wide range of sizes, so that the smaller filler particles will fill in between the larger filler particles and diatomaceous earth.

There is, of course, the choice of size of the filler particles. On the one hand, they should be as small as possible in order to approximate to line 1; on the other, they should not be so small as to enter the porous structure of diatomaceous particles. There is good reason to select particles with a size distribution similar to particles of haze that are filtered from rough beer, i.e., with a range of 0.5–4 μm (6). This will ensure that only those parts of a bed of diatomaceous earth that can accommodate beer particles will register as voidage in the test. The material used as filler in this work was Gasil 23D, supplied by Crosfield Chemicals. Figure 2 compares the particle size range of Gasil 23D with the particles in unfiltered beer. The Gasil covers the range of particles in rough beer just out of the cold conditioning tank, which are generally composed of proteinaceous material. The peak in unfiltered beer solids between 0.4 and 0.5 μm was not reflected in the Gasil, but only a small portion of these solids is removed in beer filtration.

When establishing the effective bed void volume of diatomaceous earth available for entrapment of particles, the object must be to simulate as closely as possible the packing conditions that occur when filter cake is continuously building up on a support, as during the filtration of beer. Under these conditions, filter aid is constantly supplied to the filter and a uniform bed develops with an even distribution of particle sizes throughout. This consideration becomes even more important when a fine filler is added, for if sedimentation occurs during the formation of the cake, there will be a tendency for the filler not to be trapped in the bed of diatomaceous earth but to settle out separately, and low estimates of bed void volume would result. Provided that correct packing is

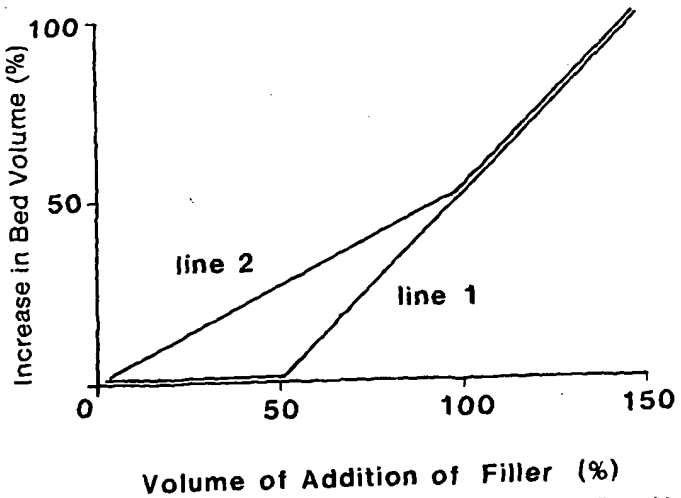


Fig. 1. Schematic representation of the increase in bed volume of filter aid on addition of filler.

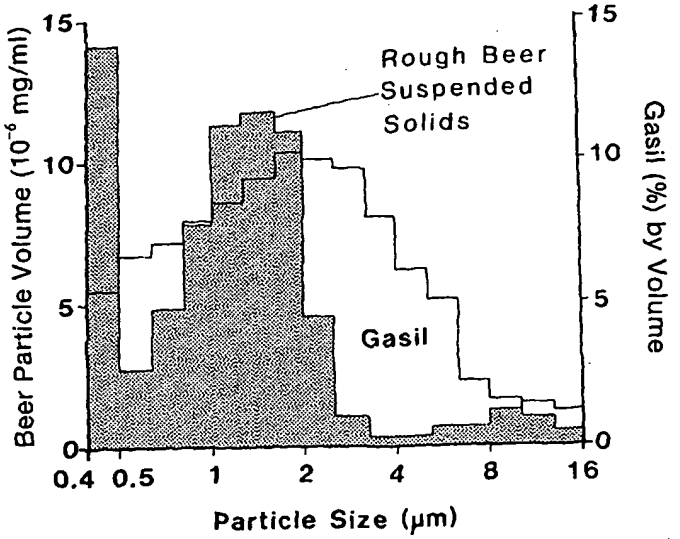


Fig. 2. Comparison of particle size profiles by Coulter Counter of Gasil 23D and suspended solids in rough beer (shown as shaded histogram).

TABLE II

Summary of Results and Comparison of True and Calculated Permeabilities

Diatomaceous Earths	Total Void Volume[a] ϵ_t (%)	Effective Bed Void Volume[b] ϵ_b (%)	Coulter Counter Particle Size[c] d_{CC} (μm)	Effective Particle Size[d] d_{eff} (μm)	Permeabilities "True"[e] (μm²)	Calculated from ϵ_t, d_{CC} (μm²)	Calculated from ϵ_b, d_{eff} (μm²)
Celite 578	83	27	7.1	11.5	0.07	6	0.025
Standard Supercel	86	35	6.8	11.5	0.20	8	0.058
Hyflo Supercel	86	58	10.7	15.4	1.62	21	1.46

[a] Measured by displacement of liquid.
[b] Measured by bed volume technique described in text.
[c] Apparent surface area weighted mean, assuming spherical particles.
[d] Calculated using equation 6.
[e] From pressure drop data.

achieved, any fluid can be used to form the bed, consequently both water and air were tested. Air has the potential to give results more rapidly, but unfortunately caused irregular packing and resulted in bed volumes larger than those obtained with water.

Measurements of Effective Bed Void Volume—Procedure

The apparatus used for these experiments was the EBC filter (2). This device was designed originally for measuring the permeability of filter sheets and filter aids in connection with equation 1.

The EBC filter comprises a pressurized glass cylinder with internal diameter 50.5 mm into which filter aid may be loaded from the top. Filter aid collects on a Whatman No. 1 filter paper that is fixed at the base of the filter and is firmly sealed onto a perforated support plate. Any liquid is extracted via a syphon connected to the apparatus beneath the support plate. In order to achieve accuracy in the bed depth measurement within 1%, a bed depth of at least 50 mm is required to accommodate the error of ±0.5 mm in depth measurement. This is equivalent to roughly 40 g of combined diatomaceous earth and filler.

The diatomaceous earth and filler cannot be added to the filter as a dilute slurry, because the rate of filtration is too slow to prevent differential sedimentation of particles of different size. The material must be made up into as thick a slurry as possible commensurate with pouring it into the filter; typically 200 ml of water per 50 g of filter aid and filler was used. As much of the filter aid and filler was scraped into the filter as possible, but no attempt was made to wash in the residual quantities, although on occasion it was necessary to agitate the slurry to remove air pockets. The filter was then sealed and top pressurized with air to between 2 to 3 atmospheres. As the materials used form a noncompressible cake, the exact pressure is of no importance. Following this procedure, no sedimentation occurred, and the water was simply squeezed from the bed. Once the filter cake formed and air had blown through, the depth of the bed was measured using a broad-ended dipstick, subtracting the distance between the top of the bed to the top of the filter from the overall depth of the filter. The residual quantity of solids not poured into the filter was dried and weighed, and the recorded depth of the cake was corrected to account for this material.

The procedure was used with 50 g of diatomaceous earth and with increasing proportions of filler. The volume of the filler per unit weight was assessed using the above procedure for 50 g of filler alone; 50 g of Gasil 23D has a bed volume of 181 ml. The volumes of filler and the bed volumes of the mixtures were expressed as a percentage of the bed volume of 50 g of the diatomaceous earth under test. A graph of percentage volume of filler versus percentage increase in bed volume was then established. The bed void volume of the diatomaceous earth was read directly off the x-axis, by back interpolation if necessary.

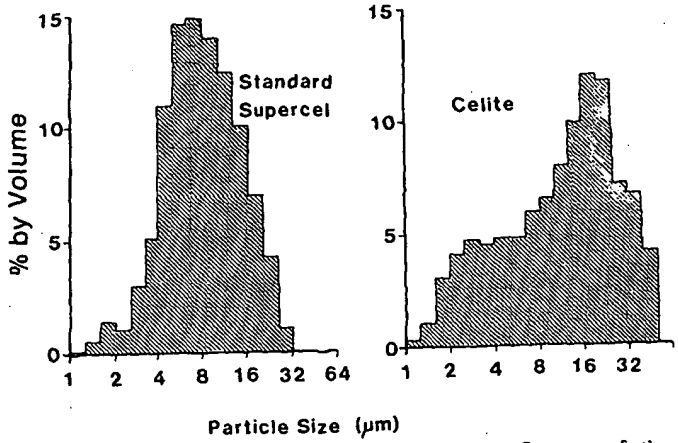


Fig. 3. Apparent particle size profiles by Coulter Counter of the Diatomaceous Earths, Standard Supercel, and Celite 578.

Miscellaneous Analyses

Coulter counting. The procedure and conditions used to count and size the suspended solids in beer (Fig. 2) were those recommended by Morris (5).

Particle sizes of filter aids and Gasil 23D. Measurements (Figs. 2 and 3) were made using suspensions of 0.1 g/L in 2.5% w/w sodium chloride electrolyte.

Bed volume. The bed volume of the filter aid was assessed following the procedure outlined in Experimental but without filler.

Density of the solid fraction of the filter aid ρ_s. A 100-ml volumetric flask was dried and weighed. Roughly 15 g of perlite or 20 g of diatomaceous earth was added as a dried powder to the flask. The flask was weighed and the exact weight of filter aid calculated. Water was added carefully to the flask until the 100 ml mark was reached. The flask was then reweighed, and the weight and volume of water were calculated. The density of the filter aid was then calculated by

$$\rho_2 = \frac{\text{Wt filter aid (g)}}{\text{100 ml} - \text{volume water added (ml)}} \quad (3)$$

Total void volume. The total void volume is the ratio of bed volume not occupied by solid material to the bed volume. It is frequently expressed as a percentage.

$$\epsilon_t = \left\{ \frac{\left[\text{bed volume} - \frac{\text{weight}}{\rho_s}\right]}{\text{bed volume}} \right\} 100\% \quad (4)$$

$$= \left\{ 1 - \frac{\rho_b}{\rho_s} \right\} 100\% \quad (5)$$

where ρ_b is the dry bulk density of the bed.

RESULTS AND DISCUSSION

The total void volume, i.e., the proportion of a bed of filter aid not occupied by *solid* material, is 86% for Standard Supercel and 83% for Celite, which are supplied by Johns Manville and are commonly used in the U.K. brewing industry. Total void volume was measured by the technique outlined in the Experimental section, and these figures were typical of those quoted by suppliers. A common method of measuring the size of diatomaceous earth particles is Coulter counting, frequently used by suppliers of filter aid. Figure 3 shows the particle size distribution for Standard Supercel and Celite 578. The mean diameters are 6.8 μm for the Standard Supercel and 7.1 μm for the Celite. Placing these figures for total void volume and particle size into equation 2 yields a value for β of 8 μm^2 for Standard Supercel and 6 μm^2 for Celite. It is assumed for the purposes of these calculations that the constant K is equal to five and that the particles are spherical. The true permeabilities derived from pressure measurements and equation 1, on the other had, are 0.2 μm^2 and 0.07 μm^2, respectively, as given in Table II. Not only are the values of β calculated using total void volume many times higher than found in practice, but the difference in permeability between the two filter aids, as calculated from equation 2, is greatly diminished. Furthermore, if total void volume is available for entrapping beer particles, it is hard to understand why so much diatomaceous earth has to be used as bodyfeed in practice.

The reason for these discrepancies is not that the well established filtration equations do not apply to brewing material but is found in the structure of diatomaceous earth. Each particle is the siliceous exoskeleton of a microscopic marine organism. While a proportion are fragments of broken skeletons, the bulk are whole units. Each unit has a hollow center connected to the outside by minute pores. In Figure 4 a whole unit is attached to a broken unit

revealing both the external and the internal structure. In most cases, the internal pores are very small and accordingly have high specific surface areas. The pores offer high resistance to the flow of liquid through them, and consequently reduce the importance of the porosity, i.e., internal void volume of the particles, in filtration. The diatom fragments also tend to be porous, and consequently behave much like complete diatoms. Thus, the effective bed void volume is equal to the total void volume of the filter aid minus the porosity of the particles. The particle porosity also means that care must be taken when interpreting Coulter Counter results.

The Coulter Counter monitors the change in electrical resistance of liquid caused by the presence of particles with a conductivity different from that of the liquid (4). It measures the number of particles and the volume of solid material in the particles. The particle diameters are calculated on the assumption that the particles are spherical and have zero porosity. Thus, with a porous structure, such as diatomaceous earth, the Counter registers only the solid fraction of the particles and consequently underestimates the true volume. It therefore follows that for the true size of a porous particle, the Coulter Counter size must be increased by an amount equivalent to the porosity of the particle.

It is these over-estimates of void volume and under-estimates of particle size and specific surface area that are responsible for the excessively high values of permeability that result from calculations based on total void volume and mean Coulter Counter size in equation 2.

Effective Bed Void Volume of Diatomaceous Earths

The results of the bed volume experiments for three diatomaceous earths are summarized in Figure 5. As stated earlier, Celite has the lowest true permeability, 0.07 μm^2, but has effectively the same mean Coulter Counter particle size of 7 μm as Standard Supercel, which has a permeability of 0.2 μm^2, i.e., three times greater. Hyflo Supercel, which is used as a first precoat, has a mean Coulter Counter particle size of 10.7 μm and high true permeability, 1.62 μm^2.

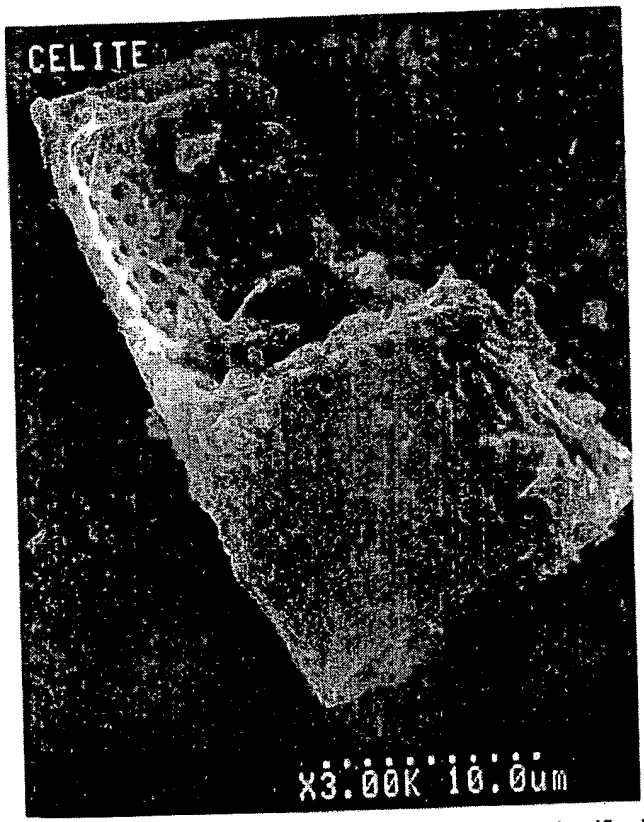


Fig. 4. Electronmicrograph of a diatom in Celite 578. Magnification × 3,000, scale length 10 μm as indicated.

The results in Figure 5 indicate effective bed void volumes of 27% for Celite, 33% for Standard Supercel, and 58% for Hyflo Supercel.

The initial gradients on Figure 5 are easily accounted for by the reasoning given earlier. The voids in the precoat filter aid, Hyflo Supercel, are sufficiently large to encompass the filler particles without any separation of the Hyflo Supercel particles. This was not true for Standard Supercel and especially for Celite, where there was considerable overlap in the particle sizes with those of the filler (Figs. 2 and 3). Some 20% of the Celite particles were similar in size to those of the filler, resulting in a marked premature bed expansion. This also indicates that the average size of the voids is smaller in Celite than in the Standard Supercel, in keeping with the generally held view of Celite's superior removal of haze material from beer. This is confirmed by the fact that Celite had 1.6 times more particles per unit weight than Standard Supercel (based on Coulter Counter figures, or calculated from the data in Fig. 3). It follows that the greater the rate of premature bed expansion for any given filler, the smaller the size of the voids.

An interesting point with Standard Supercel and Hyflo Supercel is that the rate of normal bed expansion is not unity but 0.9, indicating that some 10% of the filler was finding its way into the internal voidage of the filter aids. Considering the pore sizes in the diatomaceous skeletons and the small sizes of some of the finer filler particles, this is hardly surprising. With larger addition rates the bed expands normally.

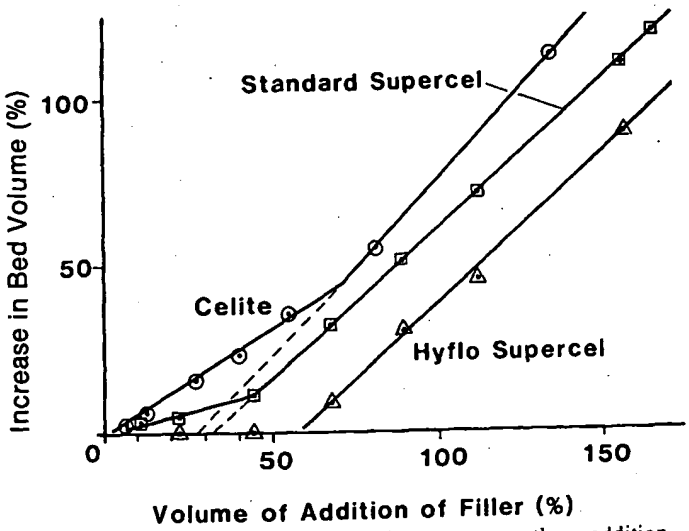


Fig. 5. Effect of increase in bed volume of diatomaceous earths on addition of filler.

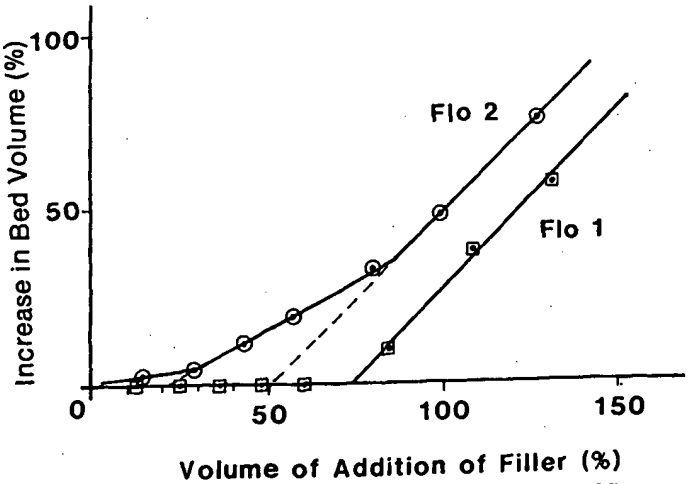


Fig. 6. Effect of increase in bed volume of perlites on addition of filler.

Effective Bed Void Volumes of Perlites

In order to demonstrate that the effective bed void volume measurement technique can be applied to filter aids other than diatomaceous earth, the results for two perlite filter aids, Flo 1 and Flo 2 supplied by British Ceca, are given in Figure 6. Flo 1, which registers an effective bed void volume of 73%, is a first precoat grade, and Flo 2, which registers an effective bed void volume of 50%, is a bodyfeed grade with a true permeability of 0.33 μm^2, similar to that of Standard Supercel.

Flo 1 registers an effective bed void volume of 73% for the entrapment of particles. This high void volume is a direct consequence of the manner in which the filter aid is produced, having a partially crushed honeycomb structure.

The data on Flo 2 (Fig. 6) suggest that there are two void volumes relevant to filtration. Flo 2 is composed essentially of platelike particles, produced by crushing the material used to manufacture Flo 1. Despite the large size of the plates, only a limited effective bed void volume of 22% has spaces sufficiently large to encompass most of the filler particles. The gradient of this section of the premature bed expansion line is less than that for Standard Supercel, indicating that these voids in the Flo 2 are larger. This was confirmed by laboratory filtration that demonstrated the Flo 2 to be less effective at removing beer haze material than the Standard Supercel. The remainder of the 50% overall effective bed void volume of Flo 2 is probably composed of space sandwiched between the plates; this void volume of 28% is considerable but appears from the gradient to be composed of very narrow spaces that are only capable of containing very fine particles.

These results on perlite further indicate that the technique developed for determining effective bed void volume has potential for the determination of size of the voids in beds of filter aid and the assessment of particle cutoff size in filtration.

Solids Loading

Because the effective bed void volume of diatomaceous earth, as measured by this bed volume technique, is so much smaller than is commonly accepted, it is worthwhile using a commonly held rule of thumb in the filtration of beer to support the results.

It is frequently claimed that 10 times more filter aid should be used than the suspended matter being removed from the beer, on a dry weight basis (3). The reason for this claim is that at higher loadings of suspended solids the bed of filter aid is progressively choked with solids, and filtration is adversely affected. If the effective bed void volume equals total voidage, e.g., 83%, the ratio of solid material to voids in the diatomaceous earth would be 17:83 or 1:5. Therefore, if one part of filter aid is used per five parts suspended matter, the bed of diatomaceous earth would just be choked. Clearly, operating with a blocked bed is impracticable and substantially more filter aid must be used. It would seem resonable to assume that increasing the dose of filter aid five times to give a ratio of 1:1 would maintain an open bed. In contrast, if the effective bed voidage is only 33%, then the ratio of porous particles to voids in the filter aid would be 2:1. If, again, it is assumed that this ratio must be increased five times to maintain an open bed when filtering suspended solids, the result would be a filter aid-to-suspended solids ratio of 10:1.

The 10:1 ratio on a weight basis also happens to coincide with a ratio of 10:1 on a volume basis for both the diatomaceous earth and filler, and diatomaceous earth and beer solids, when including the volume of water bound in the beer solids.

Experiments on a laboratory scale candle filter supported the 10:1 optimum ratio for both Celite and Standard Supercel (in terms of minimum pressure drop), despite differences in the true permeabilities of the two materials. Minimum pressures are actually obtained after a range of filter aid additions between 5:1 and 10:1 for both filter aids. The difference in effective bed void volume between Celite, 27%, and Standard Supercel, 33%, is small and had little effect on the capacity of the filter aids to entrap matter.

True Size of Diatomaceous Earth Particles

Coulter Counter sizes only correspond to the volume of the solid in the particles being measured. As diatomaceous earth comprises hollow porous particles, the size of these particles must be increased to encompass the particle porosity. The effective volume of the particles is therefore equal to the Coulter Counter volume multiplied by the cubed root of one minus the effective bed void volume divided by one minus the the total void volume, as shown in equation 6. The effective bed void volumes, indicated on Figure 5, of Celite and Standard Supercel result in this factor being approximately 5. Thus, the effective diameter of the particles is the cube root of 5, i.e., 1.7 times the measured Coulter Counter size. It is this larger measure that should be used for the purposes of estimating the size of interparticular voids and associated permeabilities in equation 2.

$$\text{Effective particle size} = \left\{\text{Coulter Counter particle size}\right\} \left\{\frac{1-\epsilon_{bed}}{1-\epsilon_{total}}\right\}^{1/3} \quad (6)$$

Mechanism of Beer Filtration with Diatomaceous Earth

Using the effective bed void volumes and the effective particle diameters derived from Coulter Counter results and particle porosity, the calculated permeabilities are 1.46 μm^2 for Hyflo Supercel, 0.058 μm^2 for Standard Supercel, and 0.025 μm^2 for Celite, as shown in Table II. These values compare with true permeabilities derived from pressure drop data of 1.62, 0.2, and 0.07 μm^2, respectively. The permeabilities calculated using the effective bed void volume for Celite and Standard Supercel are too low by a factor of about three. However, this should be compared to the calculated permeabilities using total void volume, which are between one and two orders of magnitude too large.

Permeability is critically dependent on void volume, as illustrated by the fact that small differences lead to Standard Supercel having twice the permeability of Celite, as calculated from the effective bed void volume and effective particle size. The difference between the true permeability and the permeability calculated from the effective bed void volume may be explained in two ways. Firstly, the bed void volumes measured by the bed volume measurement technique may be too small. If the permeability equation is used to calculate void volume from the true permeability, the void volumes appear to be 45% for Standard Supercel and 35% for Celite. Secondly, the void volume between particles is responsible for only part of the total permeability of diatomaceous earth, and the porosity of the particles is responsible for the remainder. Whereas the first possibility may well be partially true, the second possibility is likely to be the most important, since the data show that fine material can be washed into the internal pores of diatomaceous earth particles, indicating flow through them. In the case of Hyflo Supercel, even if the flow through the particle porosity is the same as that through the Standard Supercel particles, it is unlikely to contribute to the overall permeability of this highly permeable first precoat filter aid.

CONCLUSIONS

The effective bed void volumes of diatomaceous earth for the entrapment of suspended material in beer or other liquids may be assessed by measuring the increase in bed volume of the earth after addition of a fine filler material.

The effective bed void volume is much smaller than the total void volume measured by volumetric techniques and is approximately 30% for bodyfeed-grade diatomaceous earths.

The effective particle size of diatomaceous earths is that measured by the Coulter Counter multiplied by a factor that allows

for the internal porosity of the particles.

It appears that the relative sizes of the voids in different filter aids can be estimated by comparing the rates of premature bed expansion when using filler.

Using the effective bed void volume and the effective particle size, a much more resonable estimate of true permeability, derived from pressure drop data, can be achieved than by use of total void volume and Coulter Counter size. Further improvements in prediction await a method of accounting for flow through the porosity, i.e., the internal void volume of filter aid particles.

ACKNOWLEDGMENTS

The authors wish to thank the Director of the Brewing Research Foundation for permission to publish this paper and G. Sullivan for his technical assistance.

LITERATURE CITED

1. Coulson, J. M., and Richardson, J. F. Pages 125 and 134 in: Chemical Engineering, Vol. 2. 3rd ed. Pergamon Press: London, 1978.
2. European Brewery Convention. Page E101 in: Analytica, 3rd ed. Zoeterwoude, The Netherlands, 1975.
3. Hertjes, P. M., and Zuideveld, P. L. *Powder Technol.* 19:45, 1978.
4. Leedham, P. A., and Carpenter, P. M. *Eur. Brew. Conv. Proc. Cong., 16th, Amsterdam, 1977,* p. 729.
5. Morris, T. M. *J. Inst. Brew.* 90:162, 1984.
6. Morris, T. M. *J. Inst. Brew.* 92:93, 1986.
7. Reed, R. J. R., Grimmett, C. M., and Leeder, G. I. *Eur. Brew. Conv. Proc. Congr. 19th, London, 1983,* p. 225.

[Accepted November 18, 1986.]

APPENDIX F

Technical Information Bulletin

AL-143
Mineral Adsorbents, Filter Agents and Drying Agents

IV. DIATOMACEOUS EARTH

Known by a variety of tradenames, diatomaceous-earth products find common use as filter agents and all-purpose adsorbents, as well as in specialized applications, such as catalyst carriers. These products, also known as diatomaceous silicas, are powders generally having a silicon dioxide assay of about 90%.

The CAFA (Celite® Analytical Filter Aid) product is double-acid-washed to remove trace metal oxides and contains about 97.5% SiO_2. It is useful as an analytical filter agent and for high-purity applications.

Celite 521 is acid-washed and contains about 94% SiO_2. It is suitable for use where exceptional purity is desired.

The Celatom® products are flux-calcined, with a pH (10% slurry) of 8.0-9.5, and specific gravity of 2.33.

Table 7

Catalog No.	16,743-6	22,179-1	24,331-0	24,332-9	24,333-7	24,334-5
Grade	CAFA	Celite	Celatom FW-14	Celatom FW-50	Celatom FW-60	Celatom FW-80
Flow rate	100	200	700	2500	3000	5500
Median size (μ)	2.5	3.5	—	—	—	—
Clarity	1000	500	969	940	936	927
Acid-soluble iron (ppm)	60	15	—	—	—	—
Wet density (lb/cu ft)	17	19-90	19	18	18	17
Avg. particle size (μ)	7.5	14	8	15	19	23
Moisture (%)	1	0.5	—	—	—	—
Color	white-grey/ pink	light-pink to tan	white	white	white	white

Celite is a registered trademark of JohnsManville Corp.
Celatom is a registered trademark of EaglePicher Industries, Inc.